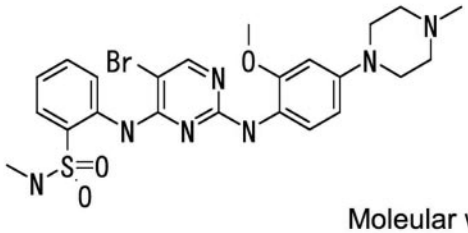
Supporting Information

Soda *et al.* 10.1073/pnas.0805381105

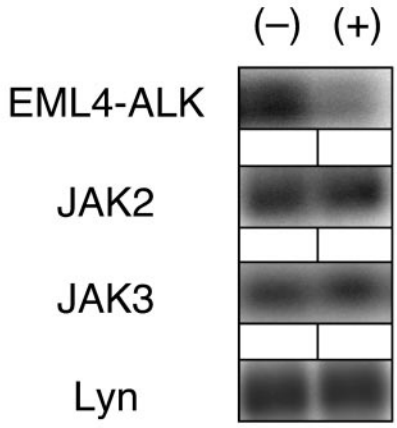
A



Moleular weight: 562.48

2-[(5-bromo-2-{[2-methoxy-4-(4-methylpiperazin-1-yl)phenyl]amino}pyrimidin-4-yl)amino]-N-methylbenzenesulfonamide

B



C

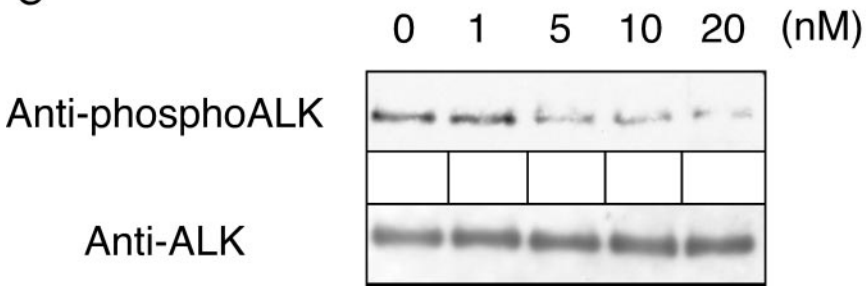


Fig. S1. High specificity of the 2,4-pyrimidinediamine derivative for ALK. (*A*) Structure of the 2,4-pyrimidinediamine derivative used in this study. (*B*) EML4-ALK, human JAK2, human JAK3, or mouse Lyn was expressed in 293 cells, immunoprecipitated with specific antibodies, and subjected to an in vitro kinase assay in a reaction mixture containing [γ-^{32}P]ATP without (Lyn) or with (EML4-ALK, JAK2, JAK3) a specific substrate and in the absence (−) or presence (+) of 5 nM 2,4-pyrimidinediamine derivative. Autophosphorylation of Lyn and phosphorylation of the YFF peptide (EML4-ALK), the peptide VLPQDKEYYKVKEPGES (JAK2), or the peptide LLPLDKDYYVVREPGQS (JAK3) were detected by SDS/PAGE and autoradiography. (*C*) Mouse BA/F3 cells expressing EML4-ALK were incubated with the indicated concentrations of the 2,4-pyrimidinediamine derivative for 6 h, after which total cell lysates (20 μg of protein per lane) were prepared and subjected to immunoblot analysis with antibodies to the Tyr-1604-phosphorylated form of ALK (*Upper*) or to ALK (*Lower*).

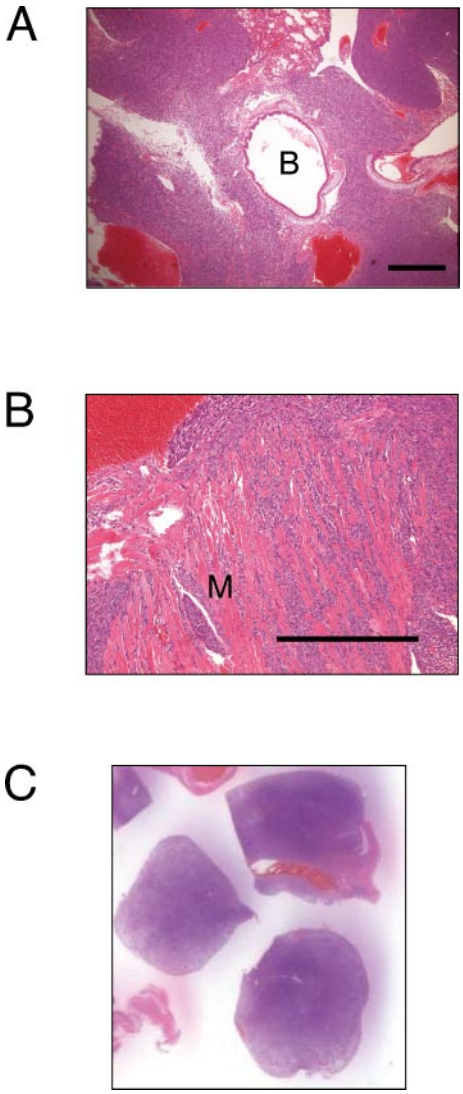


Fig. S2. Infiltration of transformed EML4-ALK/3T3 cells into tissues adjacent to the lungs. (*A* and *B*) H&E staining of tissue sections revealed massive infiltration of EML4-ALK/3T3 cells into the mediastinum (*A*) and diaphragm (*B*) of a mouse in the control group (control no. 7) described in Fig. 4. B, bronchus. M, muscle. (Scale bars, 500 μm.) (*C*) More than 90% of the lungs in this mouse were occupied with EML4-ALK/3T3 cells, as revealed by H&E staining.

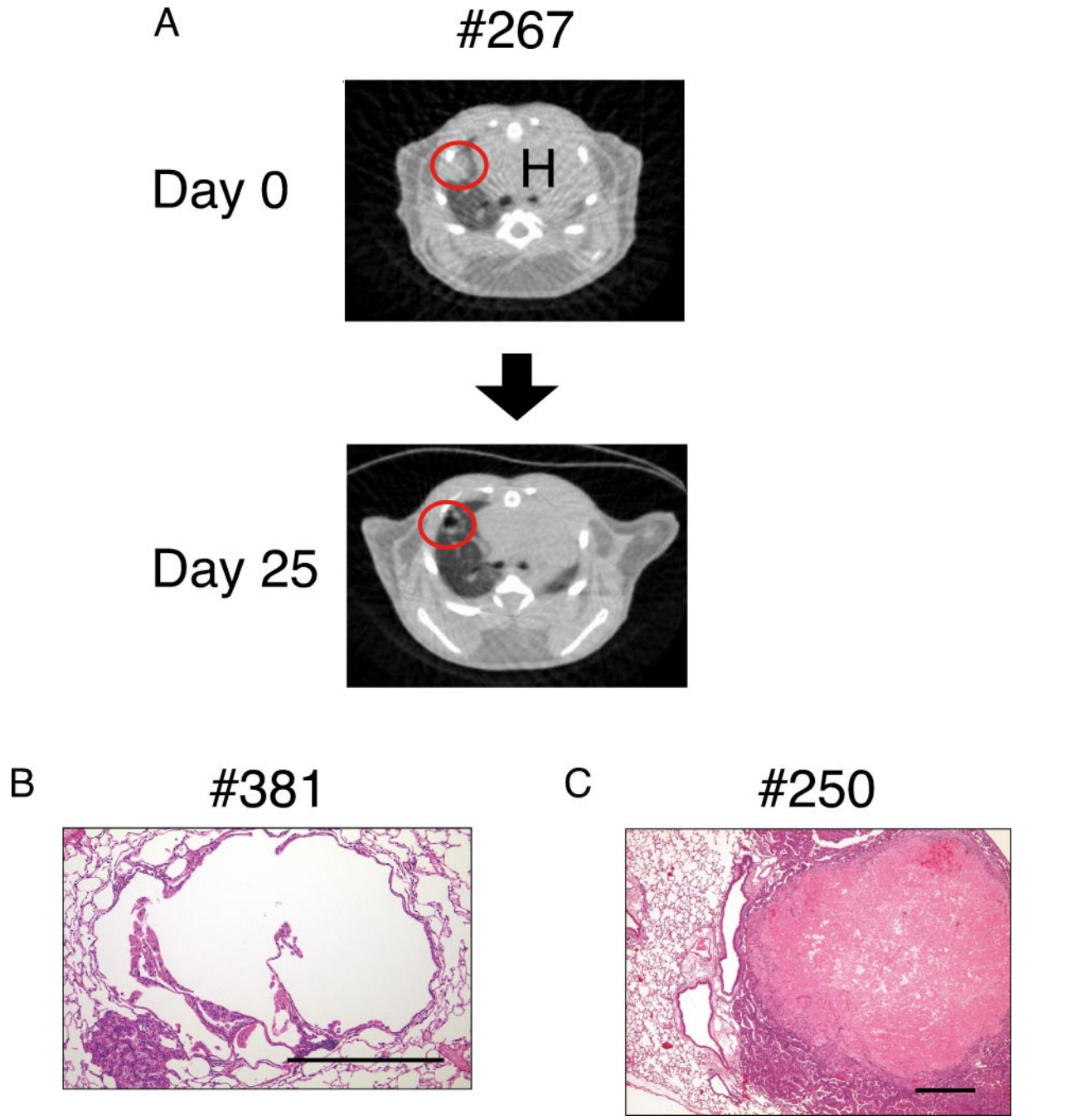


Fig. S3. Necrosis of adenocarcinoma nodules in *EML4-ALK* transgenic mice. (*A*) A transgenic mouse (ID no. 267) was subjected to CT scanning before and after treatment with the ALK inhibitor for 25 days. A large nodule in the right lung (red circle) had shrunk and become a large cyst after treatment. H, heart. (*B*) Microscopic examination of one such nodule stained with H&E after treatment in a transgenic mouse (ID no. 381) revealed the presence of a cyst surrounded by cancer cells. (Scale bar, 500 μm.) (*C*) Examination similar to that in *B* for another mouse (ID no. 250) revealed a cancer nodule to be filled with necrotic tissue. (Scale bar, 500 μm.)

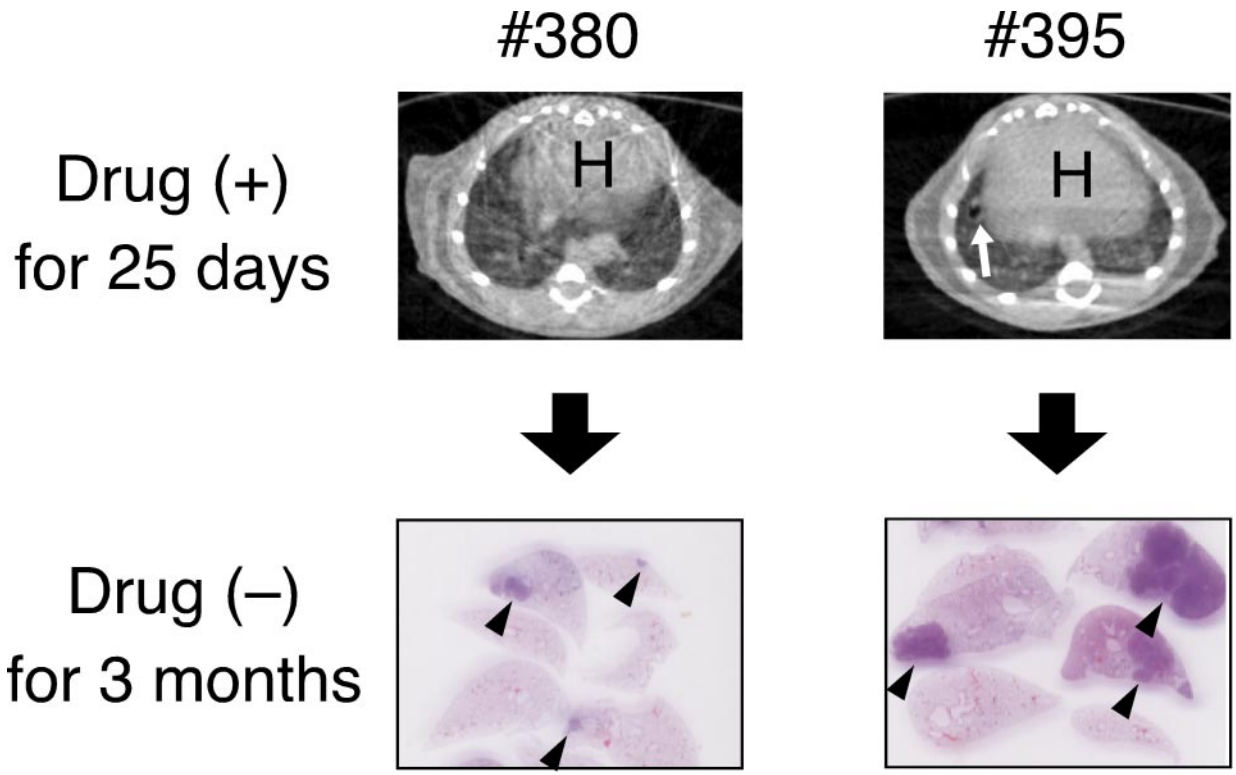


Fig. S4. Regrowth of *EML4-ALK*-positive tumors. Two transgenic mice (nos. 380 and 395) were treated with the 2,4-pyrimidinediamine derivative for 25 days, after which CT scans (*Upper*) revealed no detectable tumors in 380 and only a cyst surrounded by high-density tissue (white arrow) adjacent to the heart (H) in 395. Macroscopic examination of the lungs of these mice was performed 3 months after cessation of the drug treatment (*Lower*). Staining of the tissue with H&E revealed cancer nodules (arrowheads).

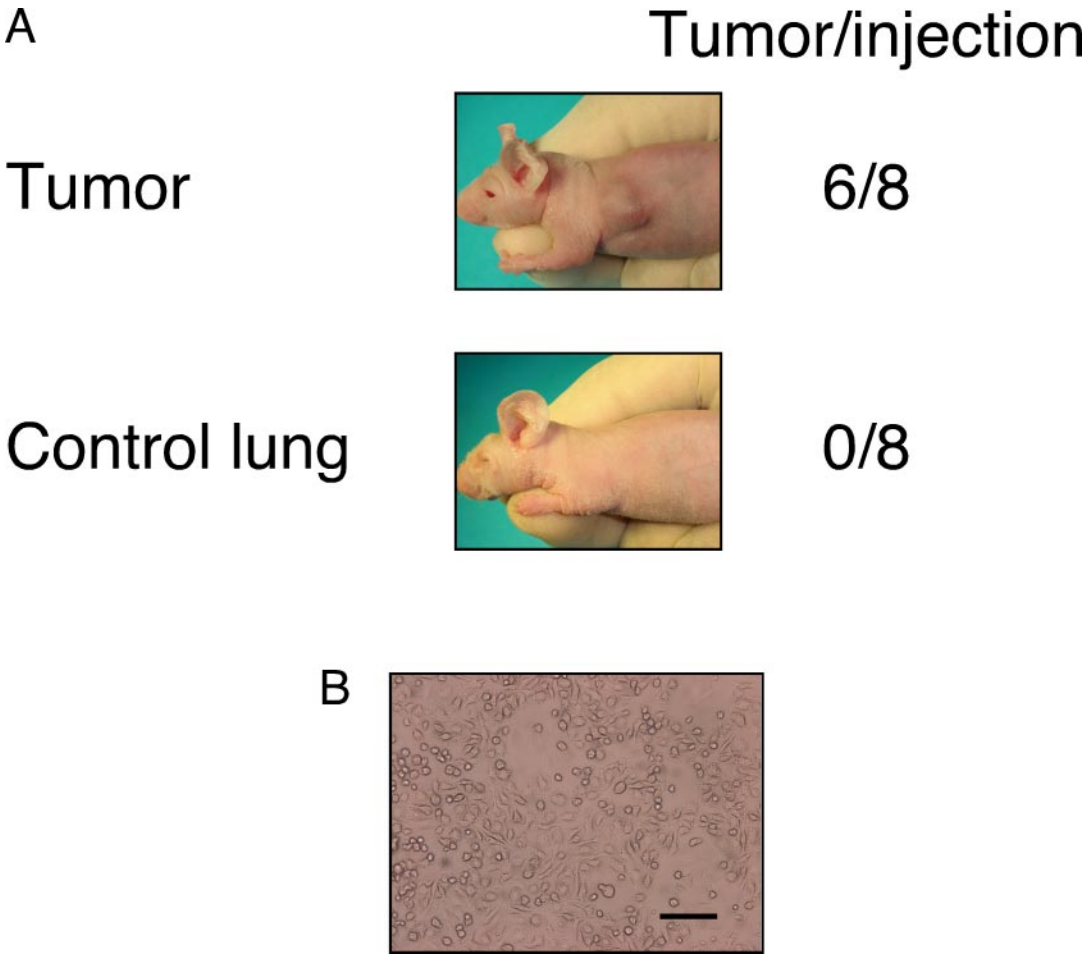
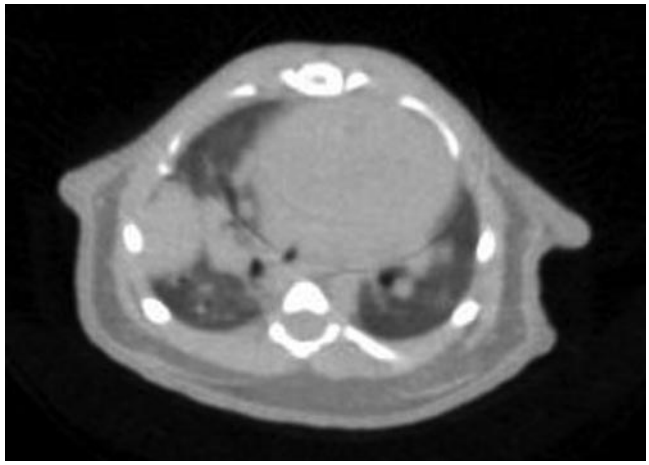
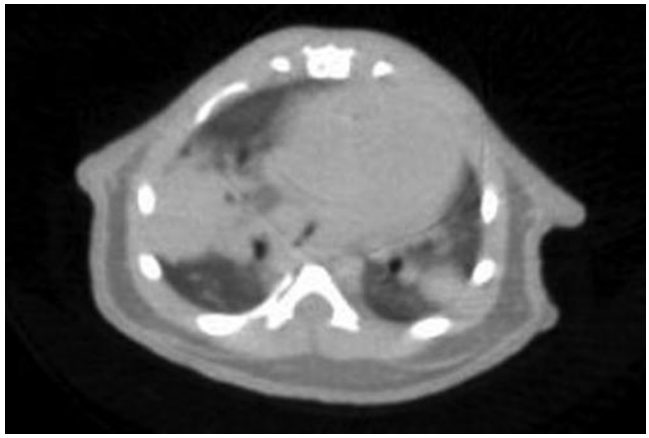


Fig. S5. Transforming phenotype of *EML4-ALK*-positive tumors. (*A*) Tumor nodules of *EML4-ALK* transgenic mice (Tumor) and lung tissue of control mice (Control lung) were resected, cut into small pieces, and transplanted s.c. into the shoulder of *nu/nu* mice. After 62 days, tumor formation was observed at 6 of the 8 injection sites in the former cohort but at none of those in the latter cohort. (*B*) Tumor nodules from *EML4-ALK* transgenic mice were also subjected to in vitro culture and have continued to grow for at least 62 days. (Scale bar, 100 μm.)



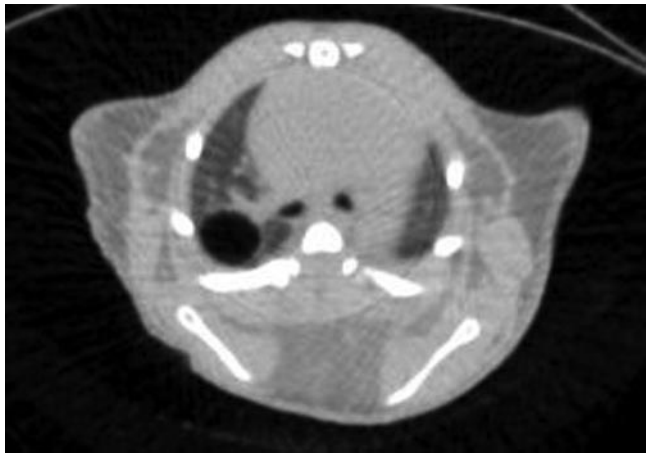
Movie S1. CT scanning at day 0 for a mouse (ID no. 367) in the control group described in Fig. 3.

Movie S1 (MOV)



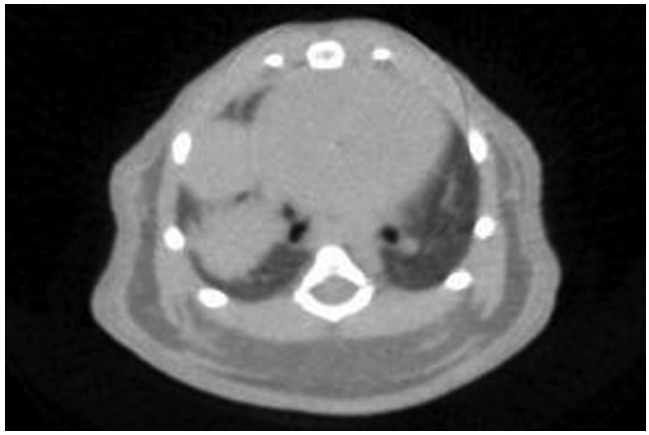
Movie S2. CT scanning at day 25 for a mouse (ID no. 367) in the control group described in Fig. 3.

Movie S2 (MOV)



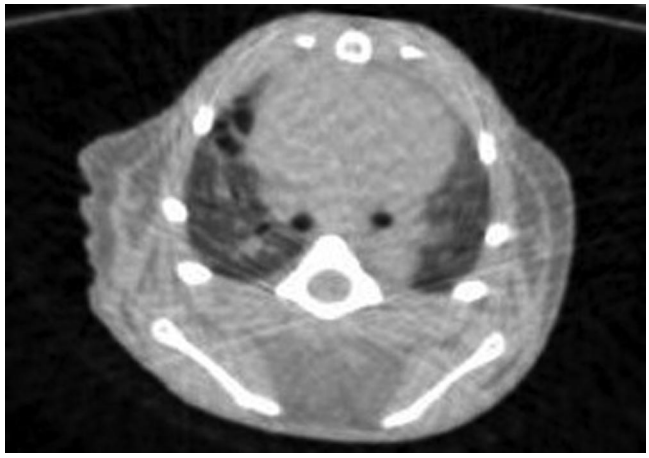
Movie S3. CT scanning at day 25 for a mouse (ID no. 373) in the treatment group described in Fig. 3.

Movie S3 (MOV)



Movie S4. CT scanning at day 0 for a mouse (ID no. 381) in the treatment group described in Fig. 3.

Movie S4 (MOV)



Movie S5. CT scanning at day 25 for a mouse (ID no. 381) in the treatment group described in Fig. 3.

Movie S5 (MOV)